# Supporting Information

**A Multi-Functional Nucleoside Probe for Studying DNA G-quadruplex Structure**

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ODN4: 5'-TA$^{8F}$GGGTTAGGGT-3'
ODN5: 5'-TAGGGTTAGGGT-3'

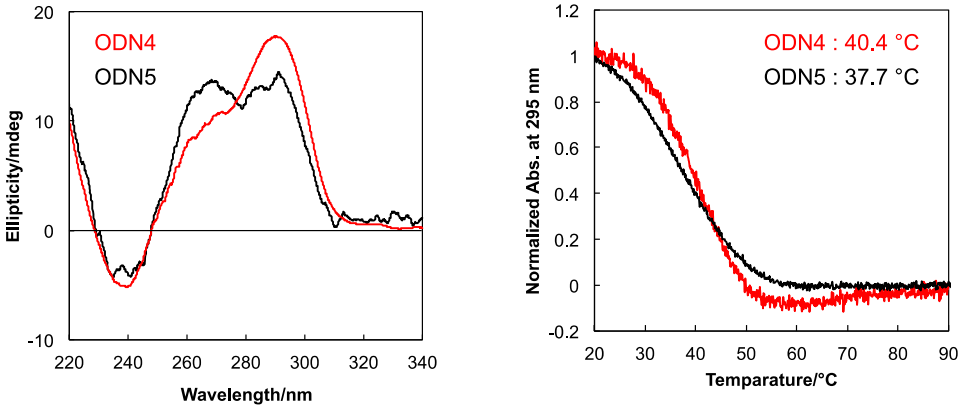



**Figure S1.** CD spectra and CD melting data of dimeric G-quadruplexes. CD data of ODN4 and ODN5 are shown in red and black, respectively. Conditions: [DNA] = 5 μM, [KCl] = 100 mM, [Tris-HCl buffer (pH 7.0)] = 10 mM.

ODN6: 5'-$^{8F}$GGTTGGTGTGGTTGG-3'
ODN7: 5'-GGTTGGTGTGGTTGG-3'

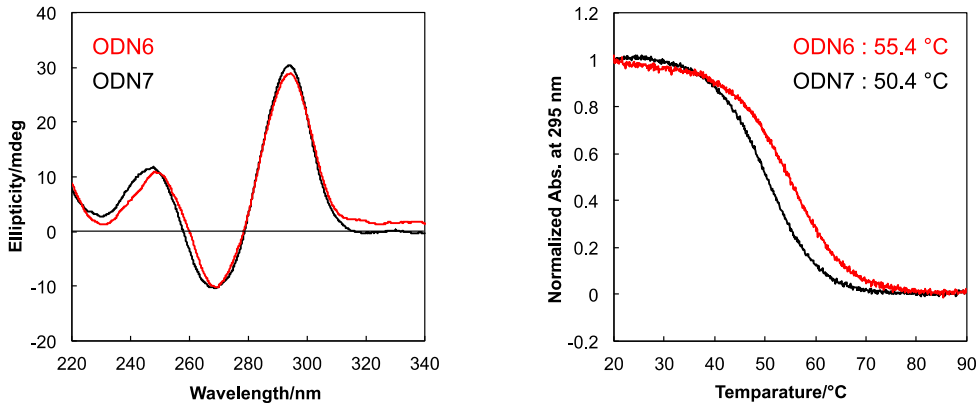



**Figure S2.** CD spectra and CD melting data of intramolecular G-quadruplexes. CD data of ODN6 and ODN7 are shown in red and black, respectively.Conditions: [DNA] = 5 μM, [KCl] = 100 mM, [Tris-HCl buffer (pH 7.0)] = 10 mM.

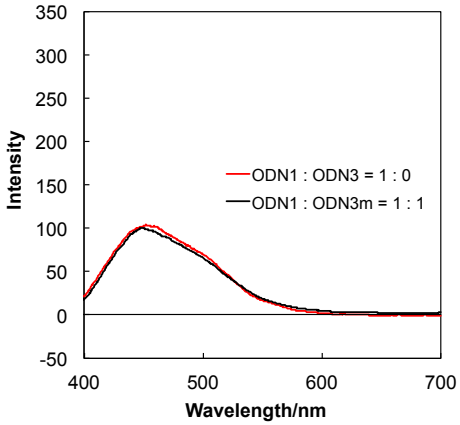



**Figure S3.** Fluorescence spectra of ODN1 only (red) and ODN1/ODN3m (black) (10 μM, 1:1 ratio) in 100 mM KCl and 10 mM Tris-HCl buffer (pH 7.0) at 25 °C ($\lambda_{ex}$ = 386 nm).

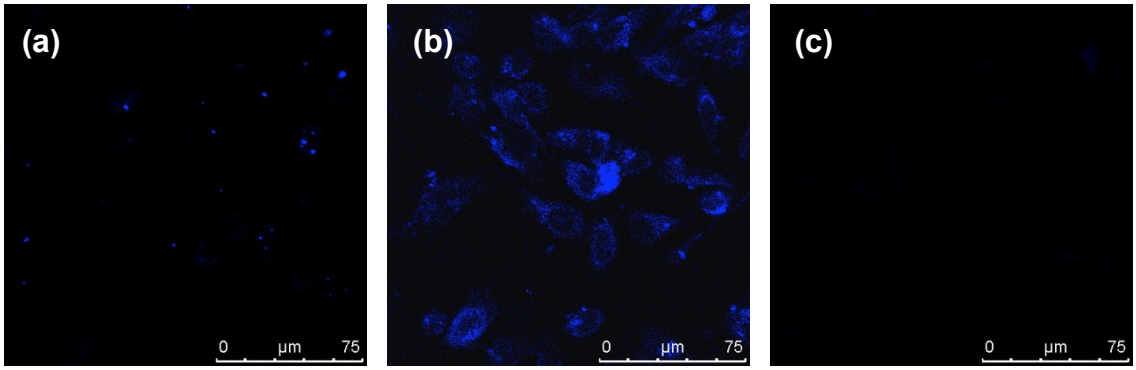



**Figure S4.** Fluorescence microscopy images of HeLa cells treated with ODN1 (a), ODN1 and ODN3 (b), ODN3 (c). HeLa cells were transfected with ODNs of 20 μM as a final concentration and incubated for 3 h. Then cells were washed twice with PBS and visualized by fluorescence microscopy (Leica TCS SP8). The excitation wavelength ($\lambda_{ex}$) and the emission wavelength ($\lambda_{em}$) were 386 and 461 nm, respectively.

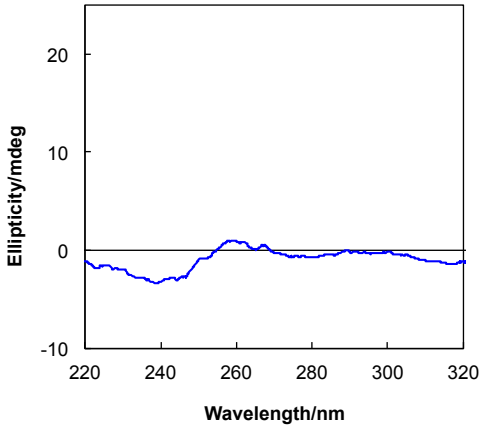



**Figure S5.** CD spectra of ODN1 (5 μM) in 100 mM KCl and 10 mM Tris-HCl buffer (pH 7.0) at 20 °C.

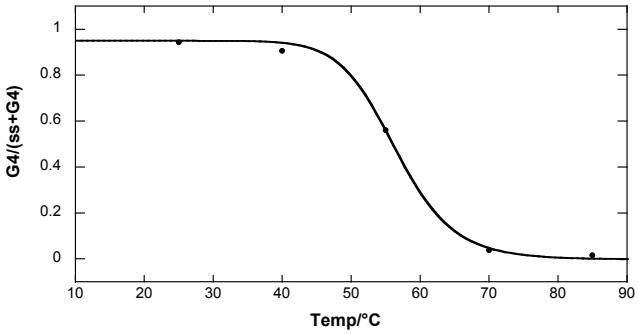



**Figure S6.** Melting profile derived from α value dependence on increasing temperature. α: the ratio of G-quadruplex (value of integral from G-quadruplex peak) divided by ssDNA + G-quadruplex (sum of value of integral from ssDNA and G-quadruplex peaks, respectively). Condition: [ODN1 and ODN3] = 100 μM, [Tris-HCl buffer (pH 7.0)] = 10 mM, [KCl] = 100 mM. The sigmoidal curve was obtained by sigmoidal curve fitting algorithm using KaleidaGraph 4.1 (Synergy Software).

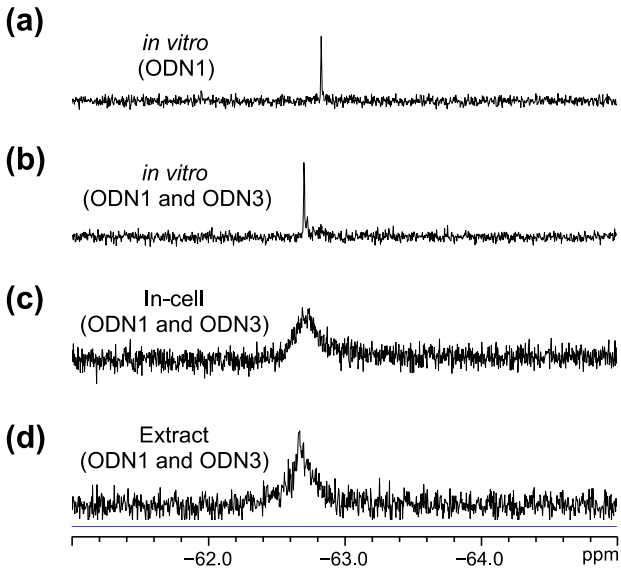



**Figure S7.** Comparison of $^{19}F$ NMR spectra of *in vitro* sample of DNA (a and b), in *Xenopus* oocytes (c) and in *Xenopus* egg lysates (d).

**Table S1** MALDI-TOF MS of all oligonucleotides used in this study. The measurement conditions were linear negative 0−3 kDa mode for ODN1 and 2, linear negative 3−10 kDa mode for ONA3, 4, 5, 6, and 7.

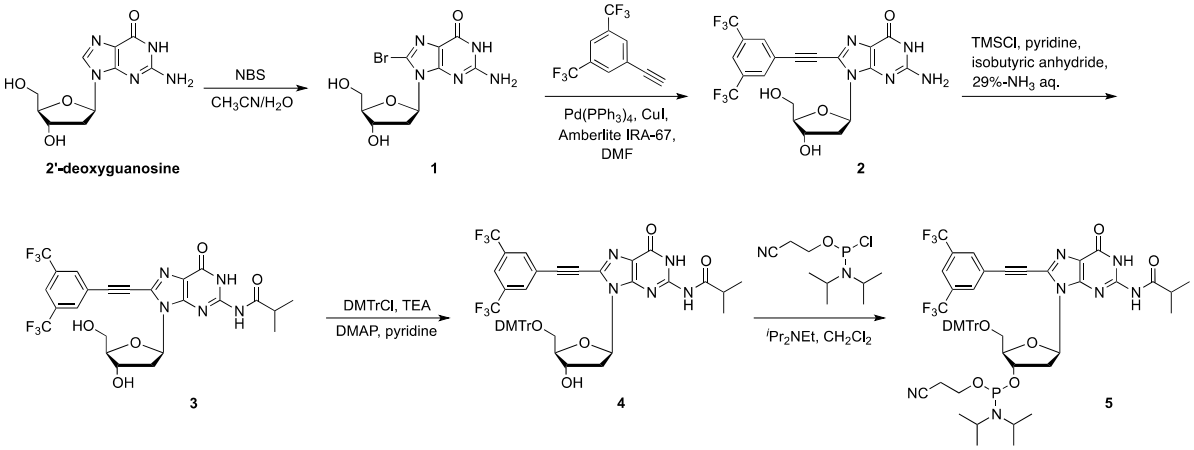
| Name | Sequences | Cacd. MS | Found MS |
|---|---|---|---|
| ODN1 | 5'-TA$^{8F}$GGGT-3' | 2082.4 | 2081.5 |
| ODN2 | 5'-TAGGGT-3' | 1846.3 | 1845.1 |
| ODN3 | 5'-GGGTTAGGGTTAGGGT-3' | 5047.3 | 5046.3 |
| ODN3m | 5'-GAGTTAGAGTTAGAGT-3' | 4999.3 | 4999.5 |
| ODN4 | 5'-TA$^{8F}$GGGTTAGGGT-3' | 3991.6 | 3991.3 |
| ODN5 | 5'-TAGGGTTAGGGT-3' | 3755.5 | 3756.3 |
| ODN6 | 5'-$^{8F}$GGTTGGTGTGGTTGG-3' | 4961.2 | 4961.6 |
| ODN7 | 5'-GGTTGGTGTGGTTGG-3' | 4725.1 | 4724.6 |

The matrix for MALDI-TOF MS was 1:1 mixture of 3-hydroxypicolinic acid (3HPA) in 1:1 acetonitrile/$H_2O$ saturated solution and 0.5 M ammonium citrate aq. solution. 1 μL of DNA sample was mixed with 1 μL of matrix solution. A spot of 1 μL of the sample-matrix mixture was placed on a MALDI target plate (MTP 384 ground steel, Bruker) and allowed to air dry at room temperature. The spectrum was measured using a matrix-assisted laser desorption/ionization-time-of-flight mass spectrometer (MALDI-TOF MS) on Bruker autoflex II mass spectrometer (negative mode) with $dT_8$ ($[M-H]^-$: 2370.603) and $dT_{17}$ ($[M-H]^-$: 5108.376) as an external standard.

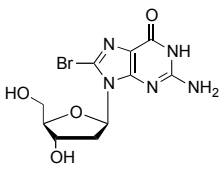
**Synthesis of $^{8F}$G phosphoramidite**

**General.**

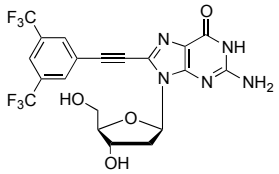
$^{1}$H-NMR, $^{13}$C-NMR, $^{19}$F-NMR and $^{31}$P-NMR spectra were recorded on a BRUKER (AV-400M) magnetic resonance spectrometer. DMSO-$d_6$ and $CDCl_3$ were used as the solvents. Coupling constants ($J$) values are given in Hz and are correct to within 0.5 Hz. Signal patterns are indicated as br, broad; s, singlet; d, doublet; t, triplet; m, multiplet. All reagents were purchased from Aldrich, TCI (Tokyo Chemical Industry Co., Ltd.) or Wako (Wako Pure Chemical Industries, Ltd.). Thin layer chromatography was performed using TLC Silica gel 60 $F_{254}$ (Merck). Compounds were visualized by staining with a potassium permanganate solution. High-resolution mass spectra (HRMS) were recorded by electrospray ionization (ESI) on a Thermo Scientific Q Exactive instrument.




**Scheme S1** Synthesis of $^{8F}$G phosphoramadite **5**

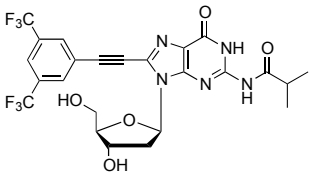


**8-Bromo-2'-deoxyguanosine (1)** 8-Bromo-2'-deoxyguanosine **1** as starting material was prepared according to previous report (A. Dumas, N. W. Luedtke, *J. Am. Chem. Soc.* **2010**, *132*, 18004). The structure was confirmed by $^{1}$H NMR and HRMS. $^{1}$H-NMR (400 MHz, DMSO-$d_6$) δ 10.79 (s, 1H), 6.49 (s, 2H), 6.16 (dd, $J$ = 7.2, 7.2 Hz, 1H), 5.25 (br s, 1H), 4.85 (br s, 1H), 4.39 (m, 1H), 3.80 (dt, $J$ = 8.4, 2.8 Hz, 1H), 3.62 (dd, $J$ = 11.6, 5.2 Hz, 1H), 3.50 (dd, $J$ = 11.6, 6.0 Hz, 1H), 3.16 (m, 1H), 2.10 (ddd, $J$ = 2.8, 6.8, 13.2 Hz, 1H). HRMS (ESI) for $C_{10}H_{11}O_4N_5Br$ $[M-H]^-$: Calcd. 344.0000; Found. 343.9981.

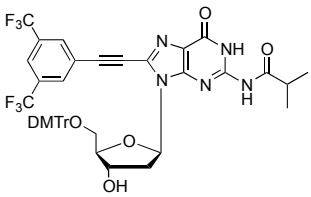


**8-[{3,5-Bis(trifluoromethyl)phenyl}ethynyl]-2'-deoxyguanosine (2)** This compound was synthesized by reference to previous report (S. R. Quake, B. M. Stoltz *et al. Chem. Commun.* **2005**, 4551). To a solution of 8-bromo-2'-deoxyguanosine **1** (1.38 g, 4.0 mmol), tetrakis(triphenylphosphine)palladium (462 mg, 0.4 mmol), CuI (152 mg, 0.8 mmol), and Amberlite® IRA-67 (4 g) in DMF (25 ml) was added 1-ethynyl-3,5-bis(trifluoromethyl)benzene (1.4 ml, 8.0 mmol). The reaction was stirred at 50 °C in oil bath. After 24 h, the

reaction mixture was cooled to room temperature, the resulting mixture was filtered through Celite, which was washed with methanol. The filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography ($CH_2Cl_2$ : MeOH = 4 : 1) to give the compound **2** (585 mg, 29%) as a yellow solid. $^1$H-NMR (400 MHz, DMSO-$d_6$) δ 10.90 (s, 1H), 8.39 (s, 2H), 8.25 (s, 1H), 6.63 (br s, 2H), 6.39 (t, $J$ = 7.2 Hz, 1H), 5.26 (d, $J$ = 4.8 Hz, 1H), 4.83 (t, $J$ = 6.0 Hz, 1H), 4.46 (m, 1H), 3.81 (m, 1H), 3.63 (m, 1H), 3.52 (m, 1H), 3.04 (m, 1H), 2.22 (ddd, $J$ = 3.6, 6.8, 13.2 Hz, 1H). $^{13}$C-NMR (100 MHz, DMSO-$d_6$) δ 155.96, 153.97, 150.87, 132.16, 131.06, 130.73, 128.06, 123.34, 117.92, 87.70, 83.53, 82.49, 70.66, 61.83, 37.60. $^{19}$F-NMR (376 MHz, DMSO-$d_6$) δ −61.42. HRMS (ESI) for $C_{20}H_{14}O_4N_5F_6$ $[M-H]^-$: Calcd. 502.0944; Found. 502.0956. Fluorescence properties: $\lambda_{ex}$ = 386 nm, $\lambda_{em}$ = 461 nm. Molar absorption coefficients ($\varepsilon$) in MeOH: $\lambda_{max}$ = 340 nm ($\varepsilon$ = 13.8 × $10^3$), 260 nm ($\varepsilon$ = 8.9 × $10^3$).

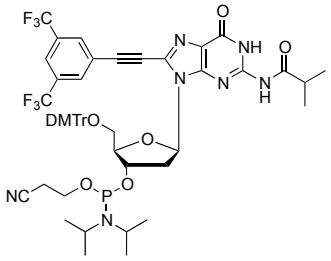



***N*$^2$-Isobutyryl-8-[{3,5-bis(trifluoromethyl)phenyl}ethynyl]-2'-deoxyguanosine (3)** To a compound **2** (750 mg, 1.5 mmol) dried three times by evaporation of pyridine (30 ml) and dissolved in dry pyridine (20 ml) was added trimethylchlorosilane (0.95 ml, 7.5 mmol). After the solution was stirred 30 minutes, isobutyric anhydride (0.84 ml, 7.5 mmol) was added, and the mixture was stirred for 3 h at room temperature. The reaction was cooled in an ice bath, and water (10 ml) was added. After 15 min, 29% aqueous ammonia (10 ml) was added, and the reaction was stirred for 15 min. The solution was then evaporated *in vacuo* and methanol (100 ml) was added to the residue. The precipitate (product) was filtered and dried, and the filtrate was concentrated *in vacuo*. The residue from the filtrate was purified by silica gel column chromatography ($CH_2Cl_2$ : MeOH = 10 : 1) to give the compound **3** (487 mg, 57%) as a yellow solid. $^1$H-NMR (400 MHz, DMSO-$d_6$) δ 12.22 (s, 1H), 11.63 (s, 1H), 8.43 (s, 2H), 8.28 (s, 1H), 6.48 (t, $J$ = 6.8 Hz, 1H), 5.30 (d, $J$ = 4.8 Hz, 1H), 4.77 (t, $J$ = 6.0 Hz, 1H), 4.51 (m, 1H), 3.84-3.81 (m, 1H), 3.62 (m, 1H), 3.51 (m, 1H), 3.12 (m, 1H), 2.81 (sep, $J$ = 6.8 Hz, 1H), 2.23 (ddd, $J$ = 3.6, 6.8, 13.2 Hz, 1H), 1.15 (d, $J$ = 6.8 Hz, 3H), 1.15 (d, $J$ = 6.8 Hz, 3H).$^{13}$C-NMR (100 MHz, DMSO-$d_6$) δ 180.19, 154.11, 148.63, 148.50, 132.44, 131.44, 131.11, 130.77, 130.44, 130.34, 126.79, 124.08, 123.39, 122.99, 121.36, 121.10, 118.65, 90.29, 87.66, 83.59, 81.80, 70.41, 61.65, 37.46, 34.71, 18.79, 18.74. $^{19}$F-NMR (376 MHz, DMSO-$d_6$) δ −61.44. HRMS (ESI) for $C_{24}H_{20}O_5N_5F_6$ $[M-H]^-$: Calcd. 572.1363; Found. 572.1371.




**5'-*O*-Dimethoxytrityl-*N*$^2$-isobutyryl-8-[{3,5-bis(trifluoromethyl)phenyl}ethynyl]-2'-deoxyguanosine (4)** To a compound **3** (487 mg, 0.85 mmol) dried three times by co-evaporation of pyridine (30 ml) and dissolved in dry pyridine (20 ml) was added 4,4'-dimethoxytritylchloride (432 mg, 1.27 mmol), triethylamine (107 µl, 0.77 mmol) and 4-(dimethylamino)pyridine (4.2 mg, 0.034 mmol). After 12 h, the solution was evaporated *in vacuo*, and the residue was dissolved in dichloromethane (50 ml) and

added aqueous 5%-$NaHCO_3$ solution. The mixture was extracted three times with dichloromethane. The organic layer was dried over $Na_2SO_4$, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (*n*-hex : AcOEt = 1 : 2) to give the compound **4** (423 mg, 57%) as a yellow solid. $^1$H-NMR (400 MHz, DMSO-$d_6$) δ 12.16 (s, 1H), 11.40 (s, 1H), 8.33 (s, 2H), 8.29 (s, 1H), 7.26-7.23 (m, 2H), 7.12-7.10 (m, 7H), 6.72-6.61 (m, 5H), 4.59-4.53 (m, 1H), 4.05-4.01 (m, 1H), 3.69 (s, 3H), 3.68 (s, 3H), 3.45 (t, $J$ = 9.6 Hz, 1H), 3.12-3.03 (m, 2H), 2.76 (sep, $J$ = 6.8 Hz, 1H), 2.44-2.37 (m, 1H), 1.14 (d, $J$ = 6.8 Hz, 3H), 1.13 (d, $J$ = 6.8 Hz, 3H). $^{13}$C-NMR (100 MHz, DMSO-$d_6$) δ 180.04, 157.79, 157.72, 154.08, 148.27, 148.07, 144.68, 135.49, 135.38, 132.33, 131.04, 130.71, 130.59, 129.57, 129.44, 127.61, 127.30, 126.29, 124.06, 123.34, 122.90, 121.41, 121.35, 112.63, 112.55, 90.52, 86.61, 85.01, 84.32, 81.73, 70.71, 64.67, 54.79, 54.74, 38.11, 34.67, 18.85, 18.58. $^{19}$F-NMR (376 MHz, DMSO-$d_6$) δ −61.39. HRMS (ESI) for $C_{45}H_{38}O_7N_5F_6$ $[M-H]^-$: Calcd. 874.2670; Found. 874.2666.



**3'-*O*-[(2-Cyanoethoxy)(diisopropylamino)phosphino]-5'-*O*-dimethoxytrityl-*N*$^2$-isobutyryl-8-[{3,5-bis(trifluoromethyl)phenyl}ethynyl]-2'-deoxyguanosine (5)** The compound **4** (530 mg, 0.61 mmol) was treated with dry *N*,*N*-diisopropylethylamine (422 µl, 2.42 mmol) and 2-cyanoethyl-*N*,*N*- diisopropylchlorophosphoramidite (405 µl, 1.82 mmol) in dry acetonitrile (10 ml) and stirred at room temperature for 2 h. After addition of dichloromethane (50 ml), the reaction was stopped by adding a 5% $NaHCO_3$ aqueous solution (50 ml). The aqueous layer was extracted three times with dichloromethane (100 ml). The combined organic layers were dried over $Na_2SO_4$ and the solvent was evaporated *in vacuo*. The residue was purified by silica gel column chromatography ($CH_2Cl_2$ : AcOEt = 3 : 1) to give the compound **5** (435 mg, 67%) as a solid. $^{19}$F-NMR (376 MHz, $CDCl_3$) δ −63.11. $^{31}$P-NMR (161 MHz, $CDCl_3$) δ 148.33, 147.84. HRMS (ESI) for $C_{54}H_{55}O_8N_7F_6P$ $[M-H]^-$: Calcd. 1074.3748; Found. 1074.3756.

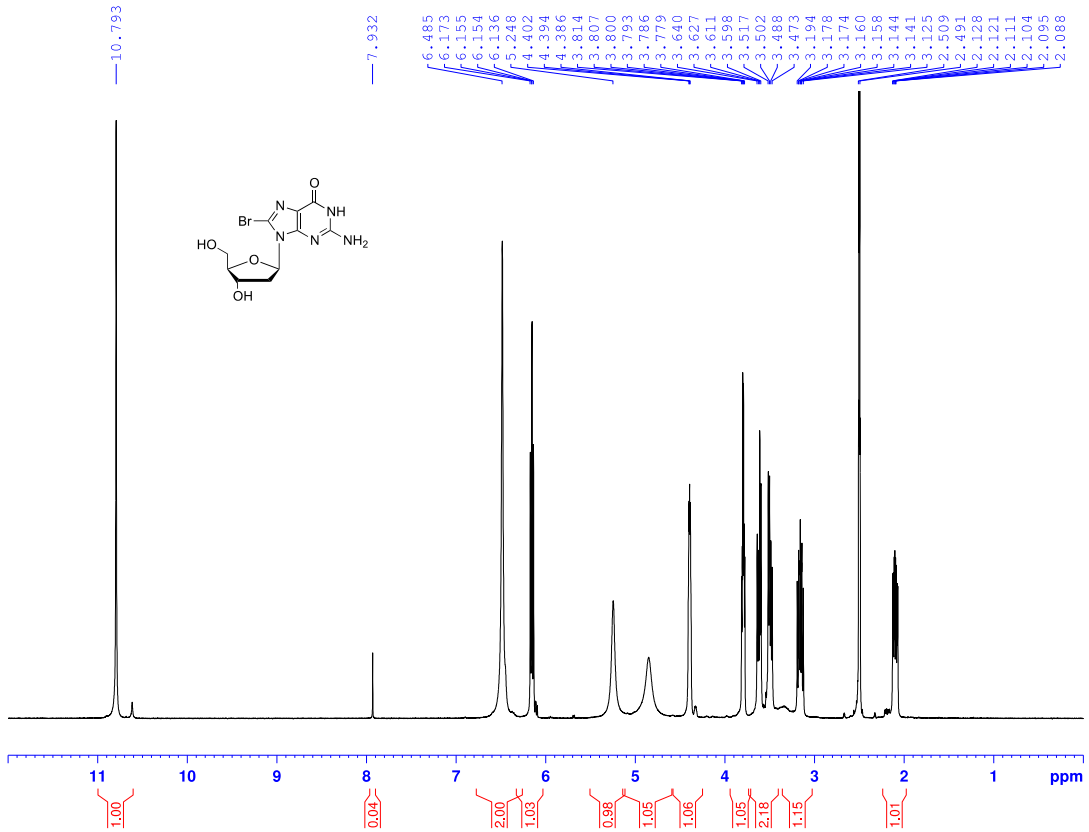



**Figure S8.** [1]H NMR spectrum of compound **1**

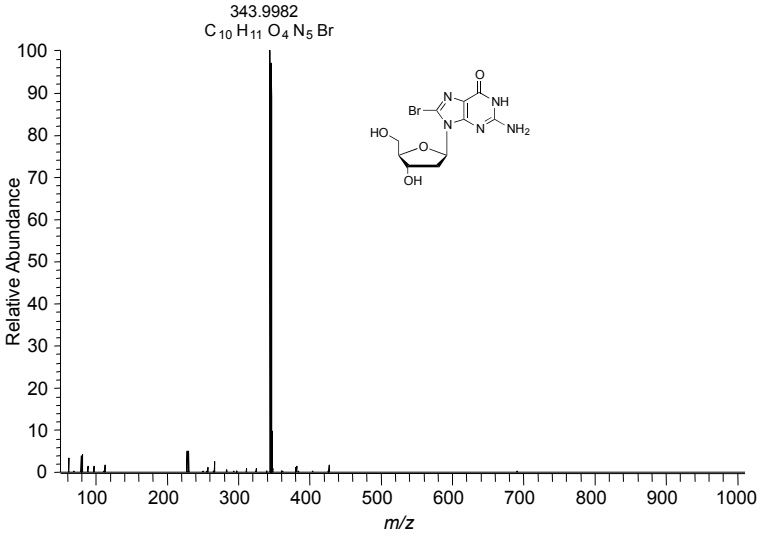



**Figure S9.** HRMS spectrum of compound **1**

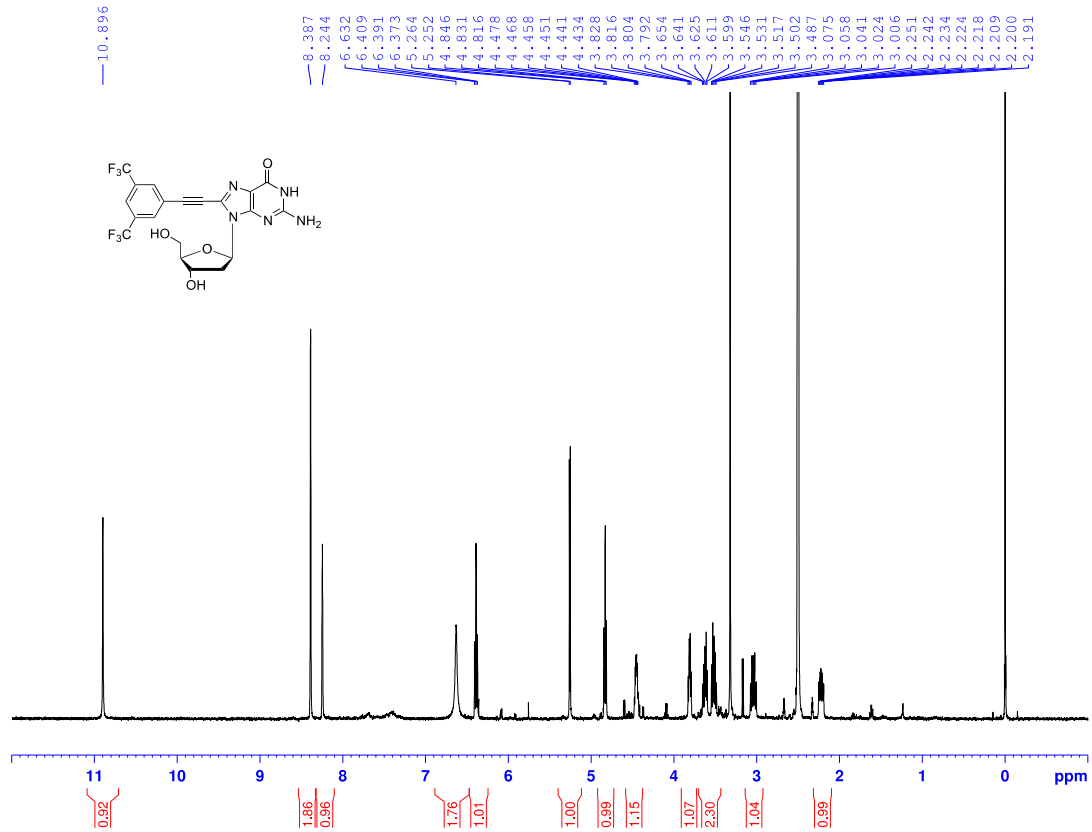



**Figure S10.** [1]H NMR spectrum of compound **2**

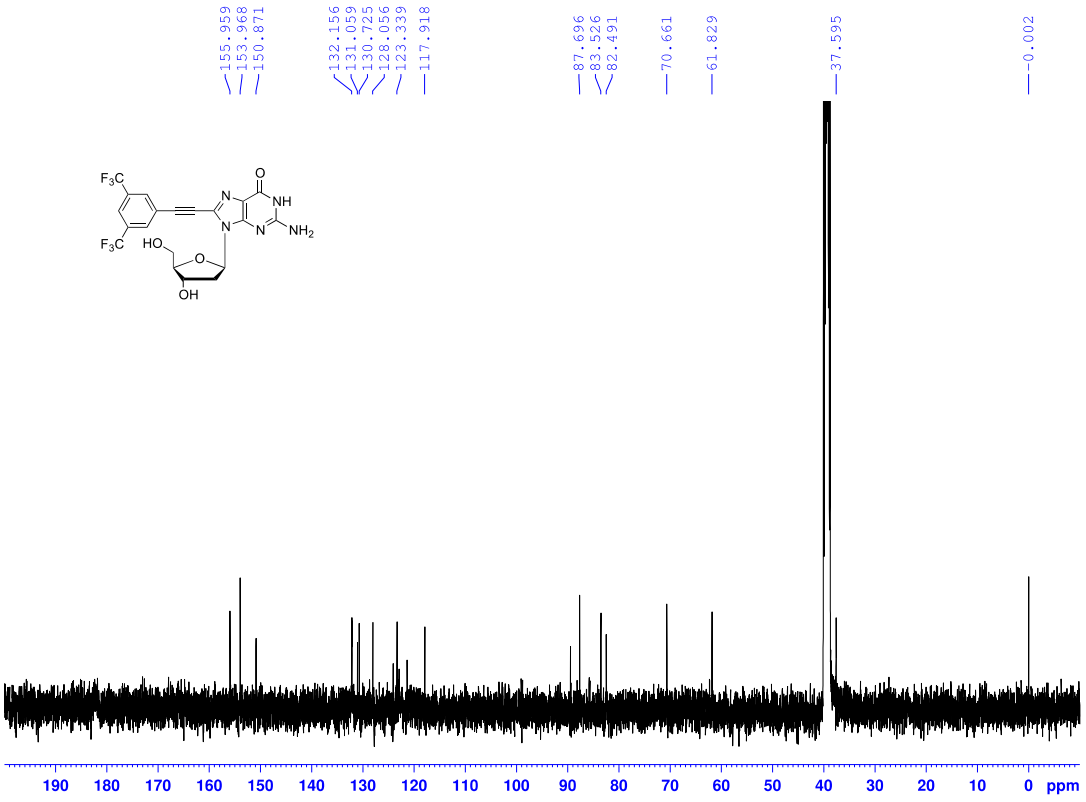



**Figure S11.** [13]C NMR spectrum of compound **2**

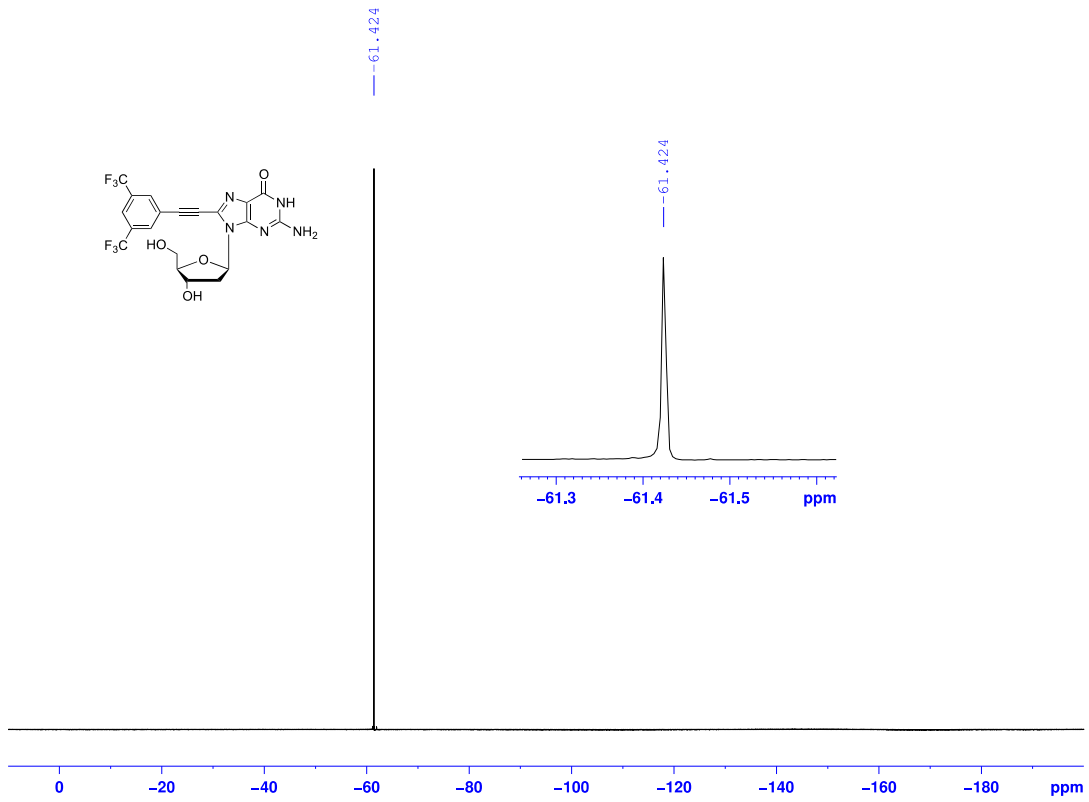



**Figure S12.** $^{19}$F NMR spectrum of compound **2**

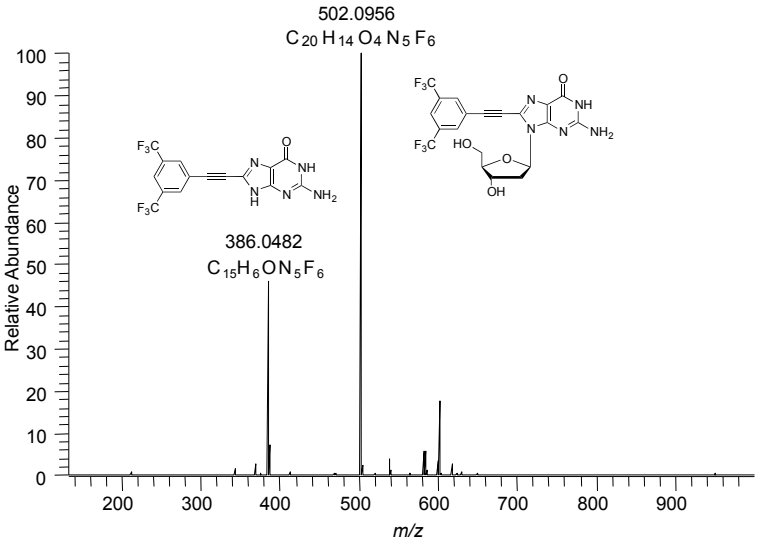



**Figure S13.** HRMS spectrum of compound **2**

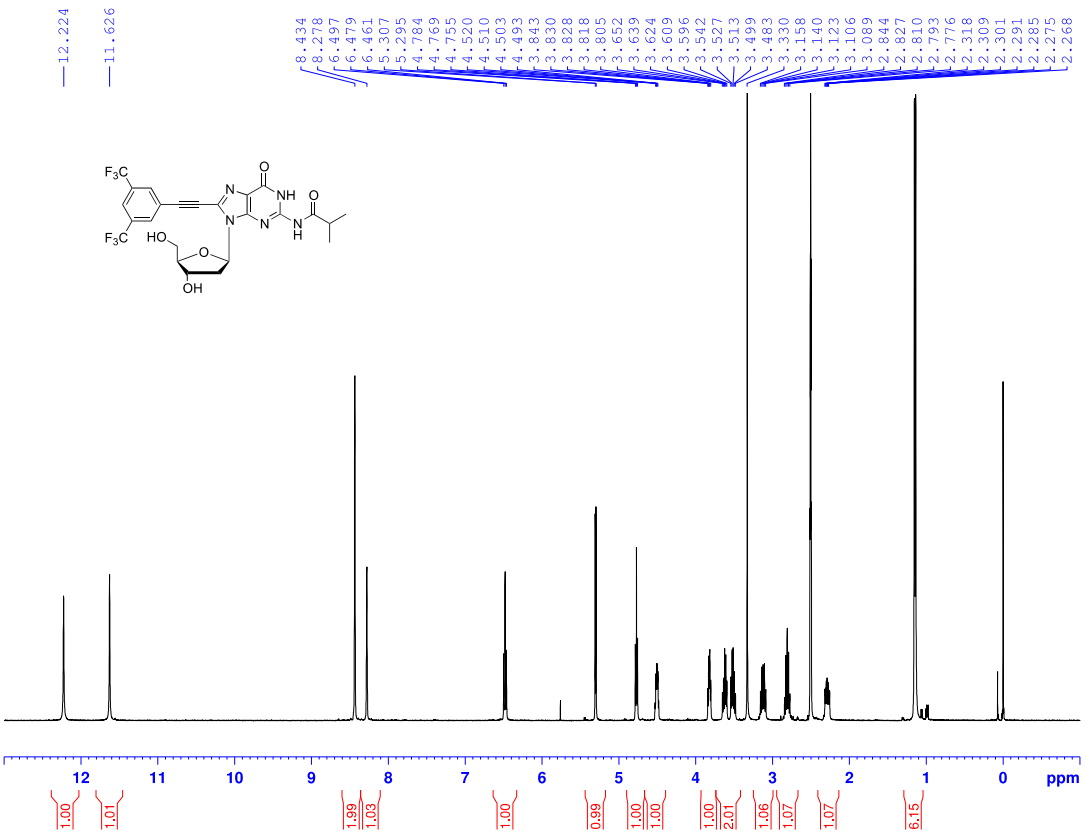



**Figure S14.** [1]H NMR spectrum of compound **3**

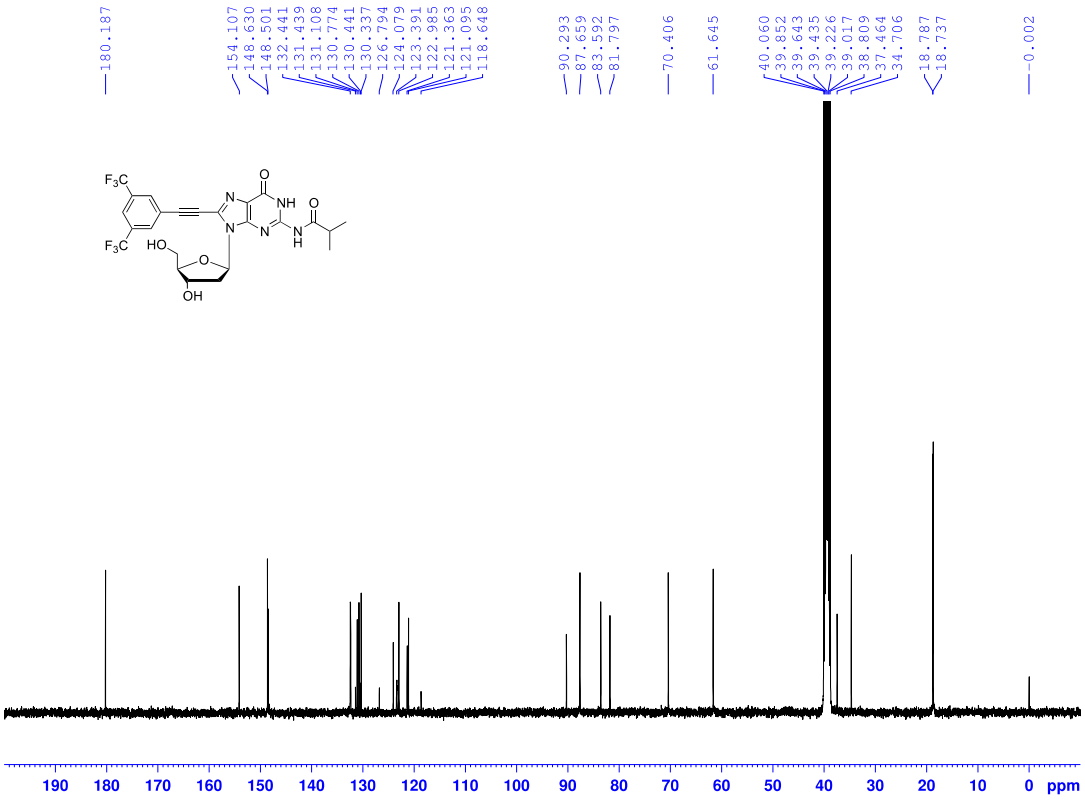



**Figure S15.** [13]C NMR spectrum of compound **3**

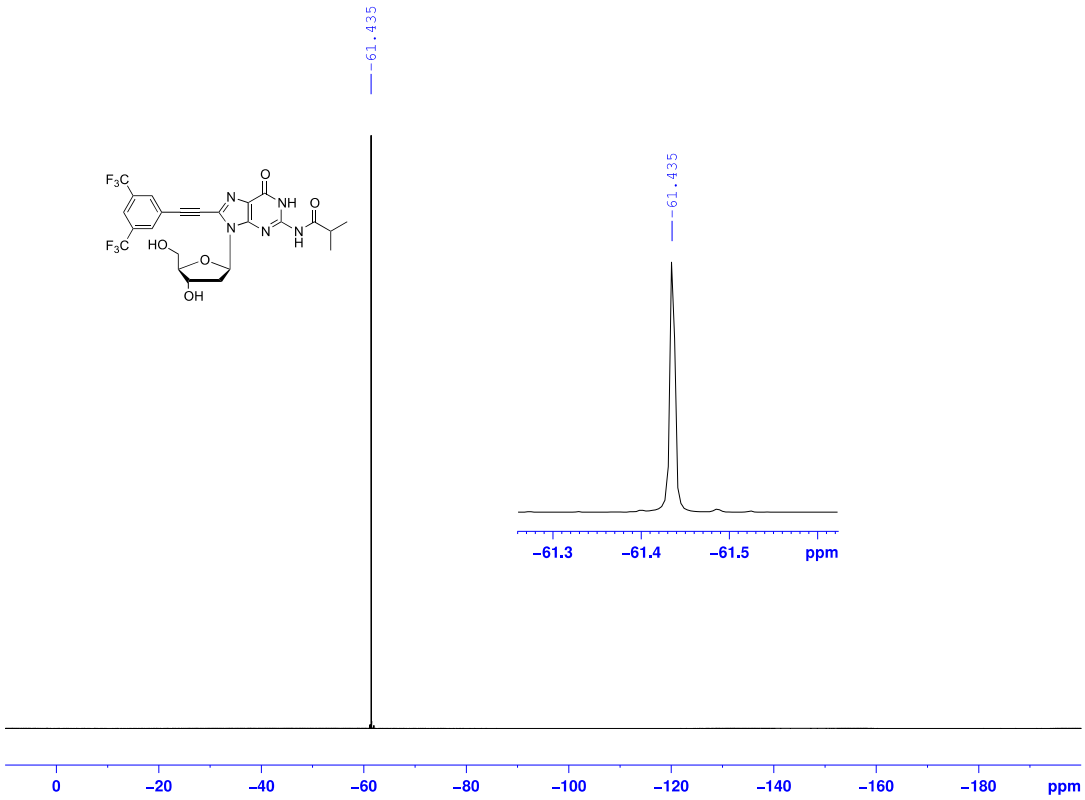



**Figure S16.** $^{19}$F NMR spectrum of compound **3**

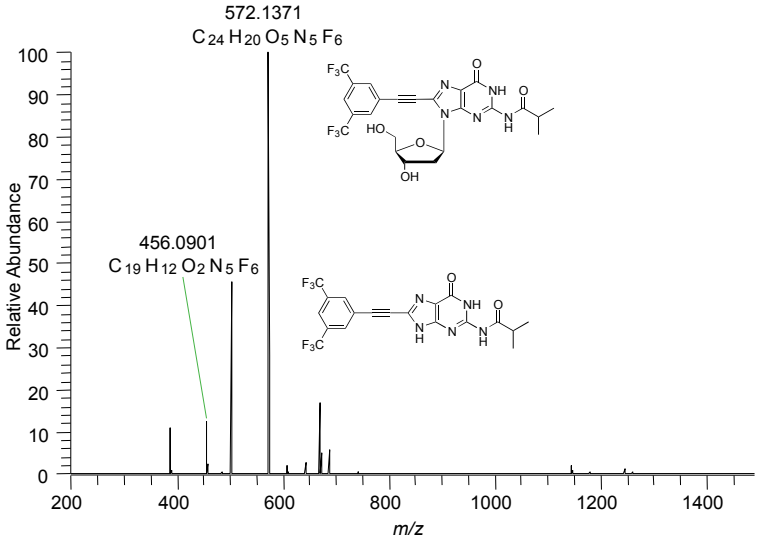



**Figure S17.** HRMS spectrum of compound **3**

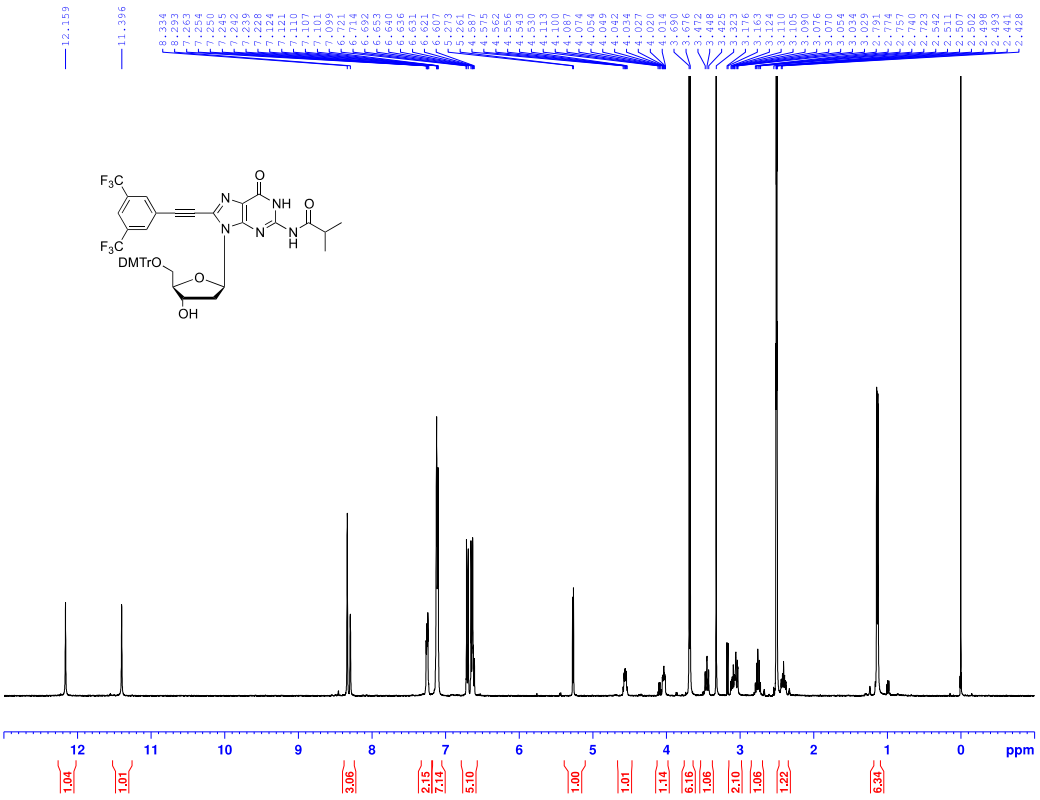


**Figure S18.** $^{1}$H NMR spectrum of compound **4**

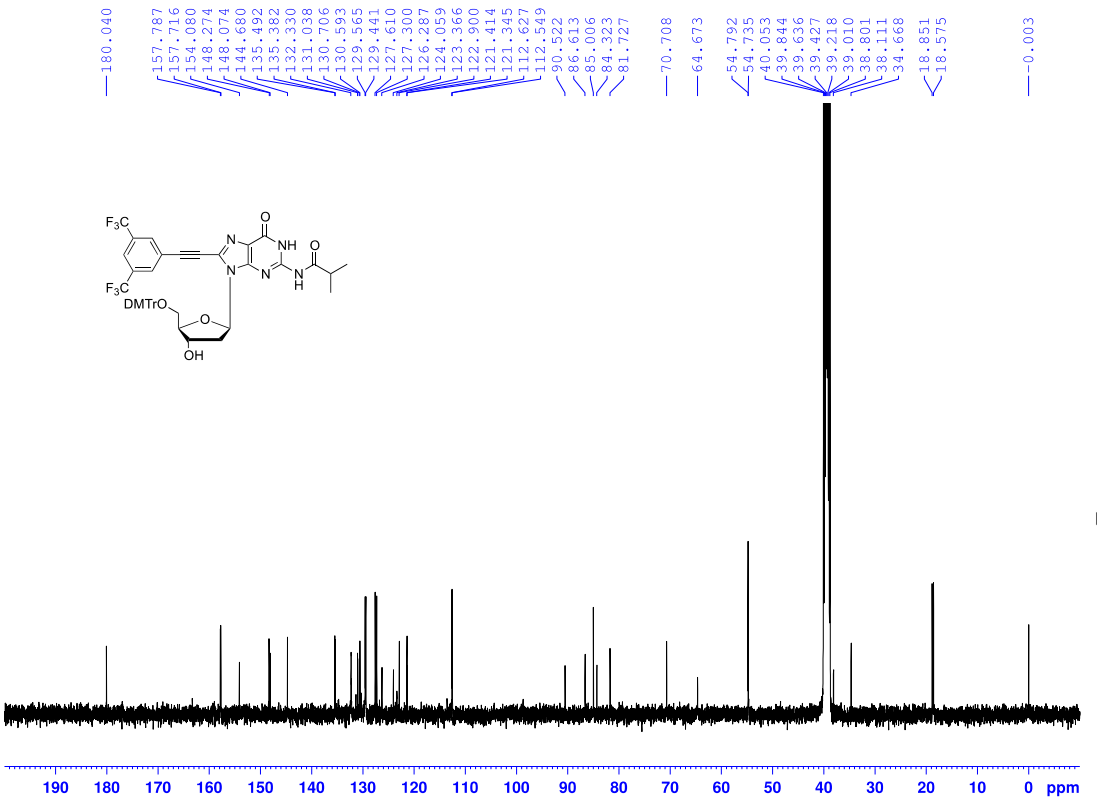


**Figure S19.** $^{13}$C NMR spectrum of compound **4**

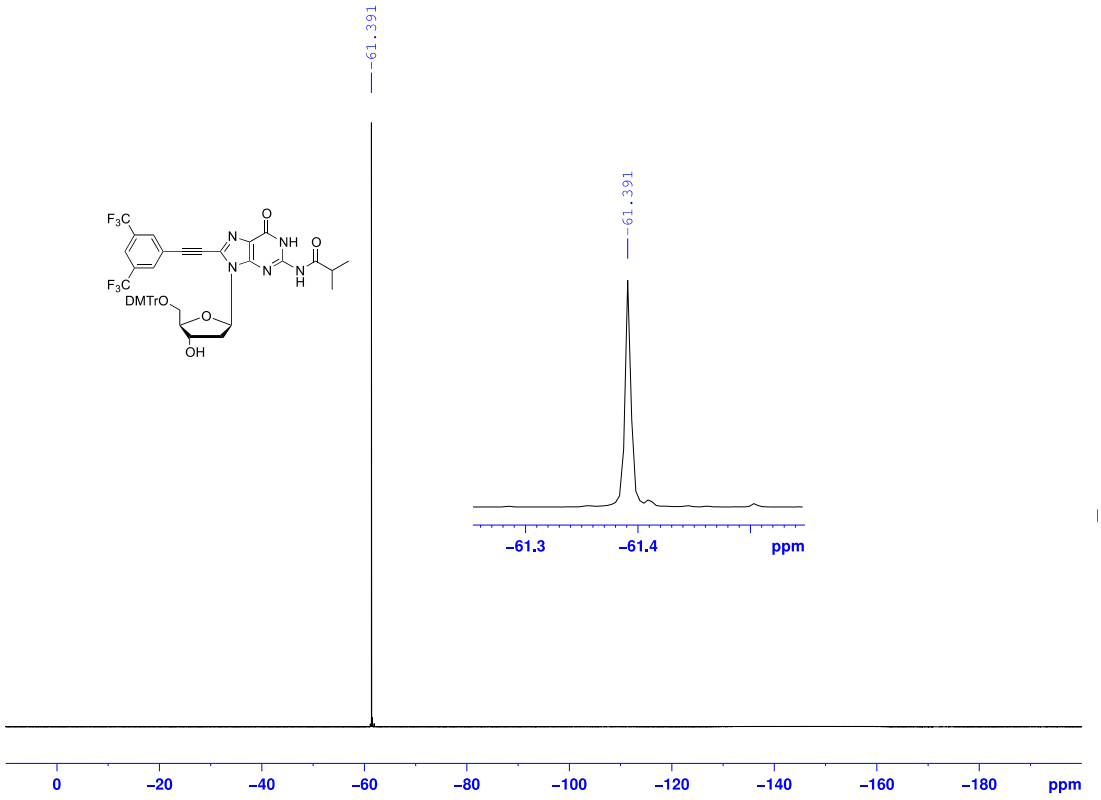



**Figure S20.** $^{19}F$ NMR spectrum of compound **4**

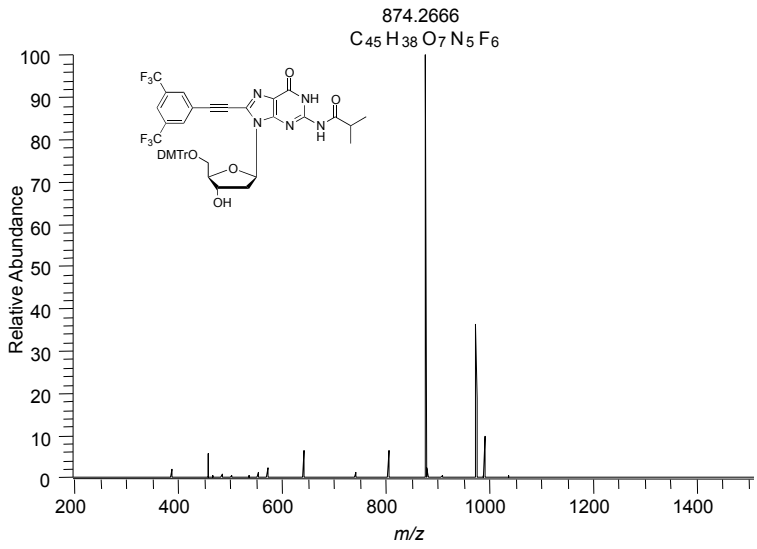



**Figure S21.** HRMS spectrum of compound **4**

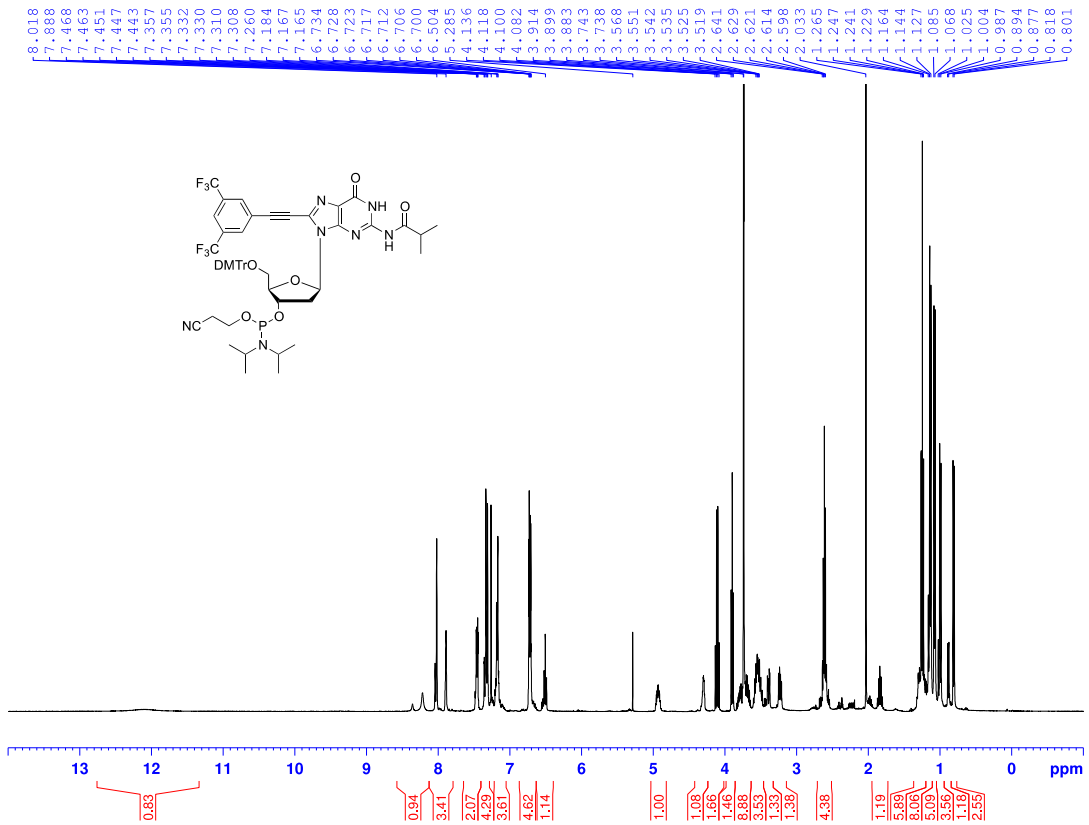


**Figure S22.** $^{1}$H NMR spectrum of compound **5**

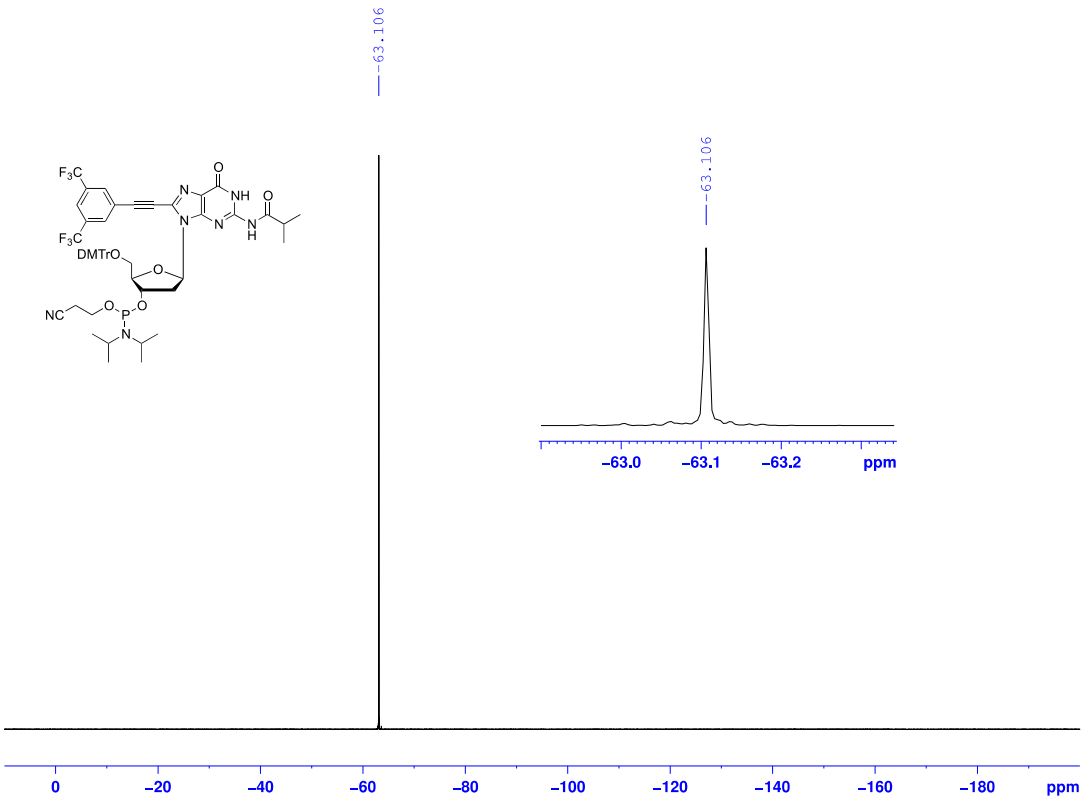


**Figure S23.** $^{19}$F NMR spectrum of compound **5**

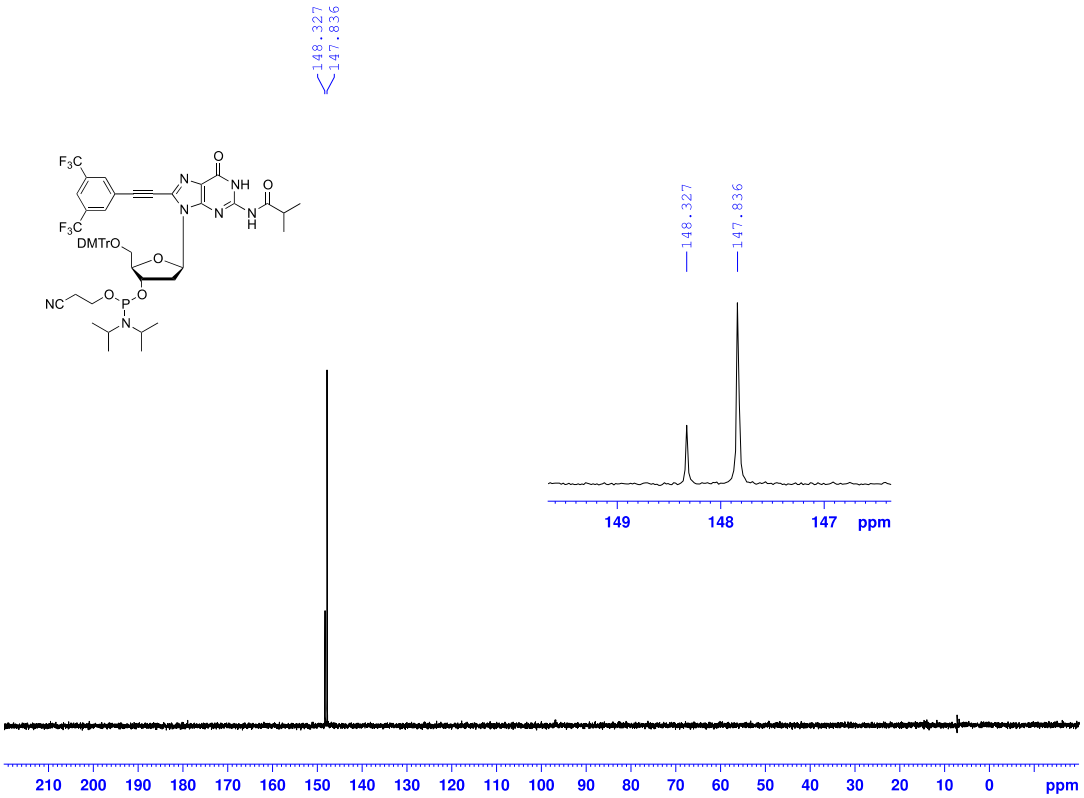



**Figure S24.** $^{31}$P NMR spectrum of compound **5**

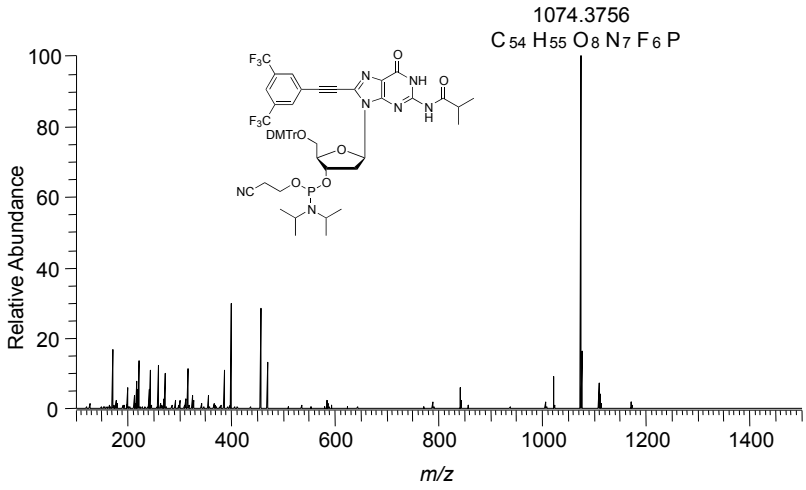



**Figure S25.** HRMS spectrum of compound **5**